

# Development and Characterization of a Hydrogel Containing Nitrofurazone for Antimicrobial Topical Applications

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**Abstract:** The goal of the research work entertained herein was the development and characterization of a poly-(vinyl alcohol) (PVA) hydrogel cross-linked with glutaraldehyde and impregnated with 0.2% (w/w) nitrofurazone (NTZ), for topical applications. To verify the active principle release capability, one has determined (i) swelling profile, (ii) *in vitro* release of NTZ via UV-VIS spectrophotometry, and (iii) antimicrobial activity via exposure to the hydrogel of ATCC strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The optimized hydrogel was further characterized via scanning electron microscopy (SEM), infrared spectroscopy with Fourier transform, moisture content determinations and thermal analyses via thermal gravimetry (TGA). Swelling tests revealed a mass increase from 100±5% up to 350±11%. Incorporated NTZ displayed bactericidal activity, as expected, being released in a linearly controlled fashion above 6 µg/mL during experiment timeframes of 14 h. SEM analyses allowed verification of a homogeneous surface morphology, while infrared spectra showed that NTZ did not bind strongly to the cross-linked polymer. Furthermore, results from thermal analyses suggested a loss of thermal stability arising from incorporation of NTZ in the hydrogel. The optimized hydrogel exhibited characteristics with high potential for (antimicrobial) treatment of skin lesions.

**Keywords:** Antimicrobial, controlled drug release systems, hydrogel, nitrofurazone, topical applications.

## 1. INTRODUCTION

Systems for the modified release of pharmaceuticals have been developed over the last few years aiming at reducing the frequency of administration, and increase both tolerance and compliance to the treatment by the patient. Among the several pharmaceutical formulations developed as modified release systems, gels and hydrogels may be considered controlled release systems for pharmaceuticals intended for topical applications, depending on both the type of formulation and intended use application [1, 2]. The topical route constitutes an interesting alternative for the administration of pharmaceuticals, due to factors such as high patient compliance rates and ease of interruption of the administration [3, 4].

Hydrogels or hydrocolloids are three-dimensional networks formed by long-chain polymers, of vegetal or animal, synthetic or microbial, synthesis. These polymers swell upon contact with water, but maintain their structural integrity [5]. Gels and hydrogels, depending on their rheological and bioadhesive behavior, may adhere to the surface of application for long periods of time, allowing the extended release

in time of the drug in the site of application, with clear advantages in terms of ease of application and/or removal. Hence, hydrogels are particularly interesting in the treatment of topical wounds due to their intrinsic low toxicity, potential for extended release of drugs, and characteristics of bioadhesiveness [6-8]. Additionally, hydrogels have been successfully used to develop oral, rectal, ocular, transdermal and implantable drug delivery systems [9-11]. Poly-(vinyl alcohol) (or PVA, for short) has been used in gels, hydrogels and polymeric films carrying drugs, owing to their properties of (bio)degradability and lack of toxicity, being considered biocompatible with living tissues in general and even with plasmatic proteins [12-15].

Hydrogels can be obtained by chemical cross-linking, aiming at modifying the properties linked to both chemical and thermal stability, structural rigidity, permeability, color, chelating efficiency and capability for both protein and cell immobilization [16]. Cross-linking consists in a type of modification aiming at joining polymeric chains, or even linking its chains to the ones of other types of polymers thus generating hybrid polymeric networks, produced via reactions between specific reactive sites present in the structural units of the polymer and certain cross-linking reagents [17, 18].

Nitrofurazone (NTZ) (among other active substances) can be used for the treatment of skin lesions. NTZ is used as topical formulations for administration of antimicrobial

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agents, as creams, gels and ointments in the treatment of infected wounds from traumatism, burns or surgical interventions [19]. NTZ is also effective in the treatment of wounds infected by significant pathogenic bacteria such as *Staphylococcus aureus* (including Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains) and *Streptococcus* [20].

In Brazil, quite recent estimates point to ca. 11.3 million inhabitants presenting some type of complications in the process of scar/healing and, of these, ca. 4 million carry chronic lesions [21]. These numbers justify *per se* the search for new resources and technologies, with lower costs and higher efficiencies. Therefore, the major goal of the research effort presented herein was to develop and fully characterize a PVA hydrogel with nitrofurazone incorporated at 0.2% (w/w) in its three-dimensional network, using glutaraldehyde as cross-linking reagent, for topical applications.

## 2. MATERIAL AND METHODS

### 2.1. Reagents

98.4% hydrolyzed polyvinyl alcohol (PVA) with a molecular weight between 146000-186000 g/mol was obtained from Vetec (Duque de Caxias/RJ, Brazil). Glutaraldehyde at 25% (v/v) was from Sigma-Aldrich (St. Louis MO, U.S.A.). Nitrofurazone (C.A.S. 59-87-0), 97.3% pure in an anhydrous base, was kindly gifted by Henrifarma Produtos Químicos e Farmacêuticos Ltda. (São Paulo/SP, Brazil). All other reagents were of PA grade or better, and the water used was purified in a Milli-Q Plus 185 system (Molsheim, France) to a final conductivity of ca.  $18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$ . The culture media used were Müeller-Hinton Agar, MacConkey Agar, Blood Agar (supplemented at the time of preparation with 5% ram's defibrinated blood) and Tryptic Soy Agar, and were all purchased from Oxoid (Basingstoke, United Kingdom). The commercial nitrofurazone disc used as reference was Sensifar® (containing 300 µg active nitrofurazone) from CEFAR (Brazil).

### 2.2. Biological Material

The microbial strains used were a kind gift from the Microbiology Laboratory of UBM (University Centre of Barra Mansa/RJ, Brazil), consisting in a strain of *Escherichia coli* (ATCC 25922, beta-lactamase negative), a strain of *Pseudomonas aeruginosa* (ATCC 27853), and a strain of *Staphylococcus aureus* (ATCC 250923, susceptible to oxacillin and penicillin).

### 2.3. Experimental Procedures

#### 2.3.1. Preparation of the Hydrogel with Incorporated Nitrofurazone

The first step in formulating the hydrogel consisted in the preparation of an aqueous dispersion of PVA at 10% (w/v). For dissolution, the dispersion thus obtained was kept under strong magnetic stirring (about 300 rpm) at  $80^\circ\text{C}\pm 5^\circ\text{C}$  during 25 min (in a magnetic stirrer from Fisatom, model 78HW-1, São Paulo/SP, Brazil), after which it was immersed in a water bath (Unique, model Ultra Cleaner, Indaiatuba/SP, Brazil) thermostatted at  $40^\circ\text{C}$  and subject to ultrasounds during

30 min for elimination of remaining air bubbles resulting from magnetic stirring. After this time period (30 min), the dispersion was allowed to stand still during ca. 20 min until reaching room temperature ( $\pm 25^\circ\text{C}$ ). The cross-linking reaction was then initiated via addition of an aqueous solution of glutaraldehyde (at 25 %, v/v). The pH was carefully adjusted to  $6.00\pm 0.05$  using 0.5 M HCl [22]. For incorporation of the active antimicrobial agent (NTZ), dissolution of NTZ in polyethylene glycol (0.2% (w/v)) was carried out prior to being added to the cross-linked PVA dispersion. The resulting solution was poured into plastic moulds and, for evaporation of the solvent, these were placed in an incubating chamber at  $60^\circ\text{C}$  (from Fanem, model 515-A, Guarulhos/SP, Brazil) during ca. 2 h, until constant weight. The final hydrogel thus obtained presented a mass of ca. 10 g with a concentration of NTZ of ca. 0.02 g. In this way, a hydrogel with 0.2 % (w/w) NTZ was produced, with a similar concentration of the ointment and topical solution Furacin®, the commercial form of nitrofurazone available in the market [23]. The hydrogel films produced were protected from the UV-light via wrapping in aluminum foil.

#### 2.3.2. Evaluation of the degree of swelling of the hydrogel

The degree of swelling of the hydrogel film was determined according to the methodology described by Mansur and colleagues [22], using the equation Degree of swelling =  $\left\{ \left( \frac{M_t - M_0}{M_0} \right) \right\} \times 100$ , where  $M_t$  represents the mass of hydrogel sample swelled after time  $t$ , and  $M_0$  represents the initial mass of dry hydrogel sample (mass of  $\pm 10$  g) [24]. With the aid of tweezers, each hydrogel sample was placed in an individual 250 mL volumetric flask with 50 mL of phosphate buffer system (PBS, 0.1 M, pH  $6.00\pm 0.05$ ) and these were maintained at a temperature of  $37^\circ\text{C}\pm 0.5^\circ\text{C}$ . Every 2 h, the mass of the hydrogel samples was determined using an analytical scale (from Tecnal, model SHI-AUY-220, Piracicaba/SP, Brazil) to assess the weight gain by the hydrogel.

#### 2.3.3. In vitro Release Assays of NTZ from the Hydrogel

The *in vitro* release assays followed the methodology described by Murphy and colleagues [8], with NTZ being determined via spectrophotometry in the UV range (wavelength of 375 nm, for a maximum absorption peak of NTZ according to Tubino *et al.* [25]). For determination of the concentration of NTZ released from the hydrogels, one has prepared a standard calibration curve using several NTZ solutions with concentrations of 0.002, 0.004, 0.006, 0.008 and 0.010 g/L of NTZ in an aqueous solution of polyethylene glycol (at 10%, v/v), with the absorbances read at 375 nm (using a UV-VIS spectrophotometer from Hach Lange, model DR 5000, Düsseldorf, Germany). Three hydrogel samples, previously dried at  $60^\circ\text{C}$  during 2 h, were individually placed in separate volumetric flasks and added with 50 mL of phosphate buffer system (PBS, 0.1 M, pH  $6.00\pm 0.05$ ). The volumetric flasks containing the hydrogel samples were duly stoppered to avoid any weight losses by evaporation, and were kept at  $37^\circ\text{C}\pm 0.5^\circ\text{C}$  in the dark. Every 2 h of incubation, the total volume of PBS buffer (50 mL) was withdrawn from the volumetric flasks and subjected to absor-

bance readings at 375 nm, using PBS as the blank, since the volume of polyethylene glycol used to prepare the hydrogel samples was virtually negligible. After every withdrawal of PBS (containing NTZ) from the volumetric flasks, a new 50 mL volume of fresh PBS was again poured into each of the volumetric flasks, and this procedure was repeated during a total timeframe of 14 h.

#### **2.3.4. Evaluation of the Antimicrobial Activity Resulting from NTZ Release from the Hydrogel in Culture Medium, by the Method of Disk-Diffusion**

NTZ release from the hydrogel was also assessed by a microbiological method, using the susceptibility test to antimicrobials, namely the disk-diffusion technique according to the National Committee of Clinical Laboratory Standards [26]. The *Escherichia coli* and *Staphylococcus aureus* strains were inoculated in TSB (Tryptic Soy Broth) broth which was added with 15% glycerol at a temperature of  $-70^{\circ}\text{C}$ . The *Pseudomonas aeruginosa* strain was kept in sterile ultrapure water, at room temperature. The *Escherichia coli* and *Pseudomonas aeruginosa* microbial strains were then plated in triplicate, both on the selective culture medium MacConkey Agar and on the non-selective culture medium Blood Agar, and incubated at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  during 24 h. The *Staphylococcus aureus* strain was plated in triplicate on the non-selective culture medium Blood Agar and incubated at  $37^{\circ}\text{C}$  during 24 h in a bacteriological incubator (from Fanem, model 515, Guarulhos/SP, Brazil). Following incubation, CFU's were aseptically collected from every strain, and were suspended in 5 mL of Müeller-Hinton broth, so as to obtain a turbidity corresponding to 0.5 in the McFarland scale, confirmed by spectrophotometric absorbance readings at a wavelength of 625 nm (spectrophotometer from Biospectro, model SP-220, Curitiba/PR, Brazil). Following confirmation of the target turbidity, inoculation in Petri dishes was performed via surface plating on Müeller-Hinton Agar. As a standard for comparing the antimicrobial activity of the pharmaceutical compound (NTZ) released from the hydrogels in the respective culture media, a commercial disk containing 300  $\mu\text{g}$  of nitrofurazone (positive control). Disks of hydrogel containing NTZ (sample) and disks of plain hydrogel without NTZ (negative control), with 7 mm diameters (similar to the commercial counterparts), were prepared via cutting hydrogel films with a sterile scalpel. The disks were placed on the surface of the agar, keeping a minimum distance of 30 mm between them (measured from their centers) and a minimum distance of 15 mm from the plate edges, thus preventing any superposition of inhibition halos. After 24 h of incubation under aerobiosis conditions and at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ , reading of the Petri dishes was carried out via visual inspection with the aid of a caliper rule, using a source of reflected light (inverted position) on a black and opaque surface.

#### **2.3.5. Scanning Electron Microscopy Analyses**

The surface of the hydrogel films containing NTZ was duly observed in a scanning electron microscope (from JEOL, model JSM-63660, Tokyo, Japan). Hydrogel samples were coated with colloidal gold under vacuum, and placed in the microscope chamber. Microphotographs were gathered using electron beams with 10 keV energy.

#### **2.3.6. Moisture Content Analyses via Infrared Heating**

For determination of the moisture content, a moisture analyzer producing infrared heating was used (from GEHAKA, model 2000, São Paulo/SP, Brazil), equipped with a programmable timer, self-calibration and temperature adjusting between  $50^{\circ}\text{C}$  and  $200^{\circ}\text{C}$ , and allowing moisture content determinations within the range 0.01% - 100%.

#### **2.3.7. Thermal Analyses via TGA**

The analytical equipment used for the thermogravimetric analyses (TGA) was from TA Instruments (model 2050, New Castle, U.S.A.).

#### **2.3.8. Analyses Via Infrared Spectrophotometry**

The infrared spectra were gathered in the range of wave-number from  $4000$  to  $650\text{ cm}^{-1}$  during 64 scans, with a resolution of  $4\text{ cm}^{-1}$ , using an infrared spectrophotometer (Illuminat IR II from Smiths, Watfords, England). FT-IR spectra were obtained using an infrared microprobe (Illuminat IR, model II, from Smiths, Watfords, England), coupled with an optical microscope (from Olympus, model BX51, Tokyo, Japan).

### **3. RESULTS AND DISCUSSION**

The hydrogels obtained according to the procedures described earlier presented characteristics of transparency, absence of air bubbles and of cracks, flexibility and uniform yellowish coloration. A moisture content of 18% was obtained, after drying at  $40^{\circ}\text{C}$  for 2 h. The low moisture content in the hydrogels with impregnated NTZ may indicate storage and handling easiness. Polyvinyl alcohol (PVA) was the polymer chosen to produce the hydrogels due to several suitable intrinsic characteristics, namely (i) a good and quick dispersion in water, (ii) mucoadhesiveness, (iii) good swelling characteristics, (iv) increased viscosity in solution, (v) ease to mould, (vi) good transparency, (vii) biocompatibility, (viii) atoxicity, (ix) hydrophilicity, (x) chemical resistance, and (xi) adsorption of proteins [18, 27, 28]. This class of polymers also favors a slow release of incorporated compounds, which is highly suitable for the treatment of topical wounds [8]. As cross-linking reagent, glutaraldehyde was selected since it permits the establishment of highly stable and irreversible cross-linked covalent bonds, allowing the material to resist to both pH and temperature changes (thermorigid materials) in a wide range of values, according to Oréface [18].

#### **3.1. Ability of the Hydrogel to Release Nitrofurazone *in vitro***

The ability of the hydrogel to release the active NTZ was assessed via determination of the swelling profile, via spectrophotometric quantification of the drug released in solution and also via a microbiological procedure through a susceptibility test to antimicrobials by the disk-diffusion technique. Regarding the swelling profile, as can be observed from inspection of Table 1, an increase in the mass of  $100 \pm 5\%$  after 2 h of incubation was obtained, which was maintained at ca.  $350 \pm 11\%$  after the fourth hour of incubation and up to the end of the 14-h timeframe of the assays. These results are in

close agreement with those reported by Murphy *et al.* [8] and Amaral [29], and indicate that the hydrogel adequately absorbed the saline (PBS buffer) solution (0.1 M, pH 6.00±0.05) and at 37°C±0.5°C. Such swelling capability is required to confirm effective polymerization and crosslinking processes, which are important phenomena to make the polymeric matrix able to incorporate the drug and produce an effective release system [29].

**Table 1. Evolution of the swelling profile of hydrogel containing incorporated NTZ.**

Time (h)	Sweelled hydrogel weight (Mt, g)	Weight gain (%)
0	10±0.5	0
2	20±0.5	100±5
4	35±0.7	350±11
6	35±0.6	350±12
8	35±0.6	350±12
10	35±0.6	350±12
12	35±0.6	350±12
14	35±0.6	350±12

Determination of the concentration of NTZ released from the hydrogel was carried out via readings of the absorbances, at 375 nm, of the supernatant solutions obtained after every 2 h of incubation of the hydrogel samples in a saline (PBS buffer) solution (0.1 M, pH 6.00±0.05). The concentration values were calculated departing from the calibration curve prepared as  $Abs@375nm = f\{[NTZ, g/L]\}$  (see Table 2 and Fig. 5). The results obtained suggest a linear release of NTZ throughout the 14 h timeframe assayed (see table 2), which will probably allow to use the hydrogel for long periods of time in dressings and/or bandages depending on the pro-

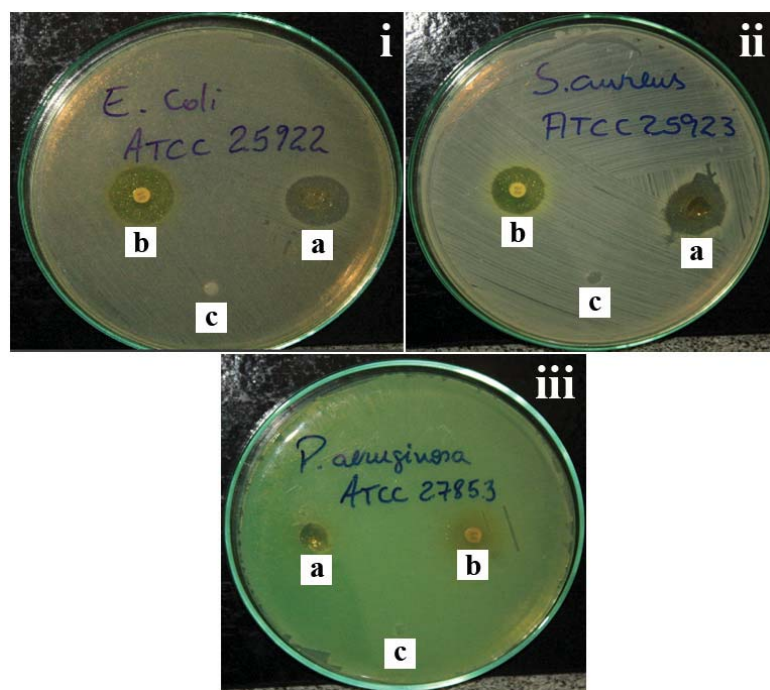
longed release of the drug, thus corroborating the results by Murphy *et al.* [8] and Amaral [29].

To shed light on the release profile of NTZ from the hydrogel, and because after the 14 h timeframe of assays the hydrogel presented a clear erosion, one decided to apply the mathematical models of Higuchi ( $Q = K_H \cdot \sqrt{t}$ , where  $Q$  is the cumulative amount of drug release,  $K_H$  is the Higuchi constant and  $t$  is time) and Korsmeyer-Peppas ( $Q_t/Q_\infty = K_{KP} \cdot t^n$ , where  $Q_t$  is the amount of drug released at time  $t$ ,  $Q_\infty$  is the total amount of drug in the hydrogel,  $K_{KP}$  is the Korsmeyer-Peppas kinetic constant,  $n$  is the diffusion or release exponent, and  $t$  is time) to the drug release data, and in fact NTZ release from the hydrogel did not occur by diffusion but by erosion of the polymeric matrix instead. This is in fact supported by the model fittings performed to the experimental (cumulative) NTZ release data, which produced a correlation coefficient  $r^2=0.92351$  for the Higuchi model and a correlation coefficient  $r^2=0.99988$  for the Korsmeyer-Peppas model. The fittings performed clearly show a poor  $r^2$  for the Higuchi model and an almost perfect  $r^2$  for the Korsmeyer-Peppas model. Additionally, the diffusion or release exponent ( $n$ ) in the Korsmeyer-Peppas model produced by fitting the model to the NTZ release data was  $n=0.9695$  (in the Korsmeyer-Peppas drug release model,  $n=0.45$  suggests Fickian diffusion,  $0.45 < n < 0.89$  suggests anomalous diffusion or non-Fickian diffusion, and  $n \geq 0.89$  suggests erosion of the polymeric chain), which clearly suggests that NTZ release occurred by a gradual erosion of the polymeric chain. The release results (see newly added Fig. 5) have thus indicated a continuous liberation within the timeframe assayed, evolving from erosion of the polymeric matrix. From inspection of Table 2, one can see that the NTZ release profile from the hydrogel was relatively homogeneous, allowing one to conclude that in liquid medium an offer of constant concentrations of NTZ does occur throughout time, at least during the timeframe assayed (14 h). This is especially important when one deals with antimicrobial activity, since any oscillations of concen-

**Table 2. Evolution of the NTZ concentration released from the hydrogel throughout a 14-h timeframe, in a saline (PBS buffer) solution (0.1 M, pH 6.00±0.05) [Calibration curve for Absorbance at 375 nm vs. NTZ concentration:  $Abs = 43.0034 \times [NTZ, g/L] + 0.04178$ ;  $r = 0.99543$ ].**

Time (h)	Average (n=3) Abs at 375 nm	[NTZ], g/L	NTZ (g/L) released from the hydrogel	NTZ released (%) relative to the total incorporated in the hydrogel
0	0.000	0.00000	0.00000	0
2	0.338	0.00688	0.00688	1.72
4	0.328	0.00665	0.01353	3.38
6	0.325	0.00659	0.02012	5.03
8	0.324	0.00656	0.02668	6.67
10	0.319	0.00645	0.03313	8.28
12	0.308	0.00620	0.03933	9.83
14	0.281	0.00563	0.04496	11.24



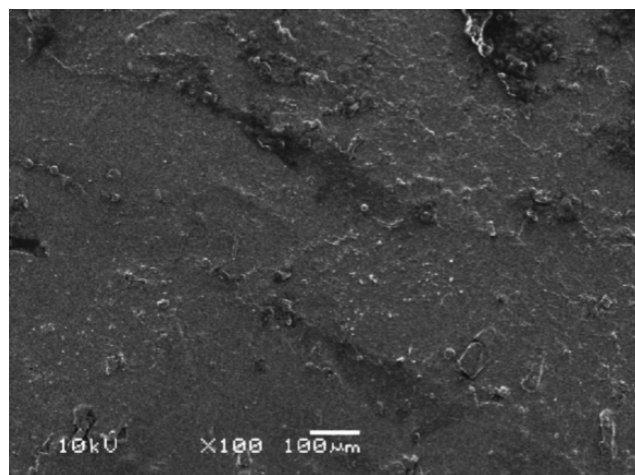


**Fig. (1).** Macroscopic evaluation of the susceptibility test to nitrofurazone of (i) *Escherichia coli* ATCC 25922, (ii) *Staphylococcus aureus* ATCC 25083, and (iii) *Pseudomonas aeruginosa* ATCC 27853 ([a]: hydrogel disk of cIPVA/Glut-NTZ; [b]: commercial NTZ disk; [c]: hydrogel disk of cIPVA/Glut).

tration in the biophase have the potential to be correlated with the selection of antibiotic-resistant strains. According to Yilmaz *et al.* [30], the minimum inhibitory concentration (MIC) of NTZ for the bacterial pathogen strains tested is, respectively, 32  $\mu\text{g/mL}$  for *Escherichia coli* and 4  $\mu\text{g/mL}$  for *Staphylococcus aureus*, while *Pseudomonas aeruginosa* is resistant to NTZ. The (calculated) NTZ concentrations released from each hydrogel sample were higher than 5  $\mu\text{g/mL}$  at all times. Hence the concentration of antimicrobial agent released is above the MIC for *Staphylococcus aureus*, one of the major bacterial pathogens responsible for infections in topical wounds. The activity of NTZ released from the hydrogel was evaluated via the disk-diffusion method (see Fig. 1), by measuring the inhibition halo of bacterial growth. For the cultures of *Escherichia coli* and *Staphylococcus aureus* (see Figs. 1i, ii), the results from the measurements of bacterial growth inhibition halos with hydrogel disks incorporating NTZ were, respectively, of 18 and 21 mm. The diameters of reference inhibition halos, according to CLSI [26], must be higher than 17 mm. Therefore, our results clearly demonstrate that the bactericidal activity of NTZ against *E. coli* and *S. aureus* was not modified due to being released from the hydrogel. For the *Pseudomonas aeruginosa* strain (see Fig. 1iii) one did not detect any growth inhibition, evidenced by the absence of inhibition halo in all hydrogel disks, which can be ascribed to its natural resistance to NTZ, according to Yilmaz *et al.* [30]. The agar diffusion method relies on the diffusion of the compound tested through water-containing agar medium. The diffusion of the drug is dependent upon the size, shape and polarity of the antibacterial material; chemical structure and the cross-linking level of the film [31]. Thus, it was important to confirm the antimicrobial properties of the newly developed hydrogel.

### 3.2. Physico-chemical Characterization of the Hydrogel

The surface morphology of the hydrogels produced was further analyzed by scanning electron microscopy (see Fig. 2). One can easily realize from inspection of Fig. 2 that the hydrogel matrices produced exhibited a uniform morphology. Such surface structure facilitates solvation and dissolution upon contact with the solvent. Thus, upon contact with the moisture from the skin, especially with the characteristic tissue discontinuity observed in wounds, the hydrogel produced by us may allow the release of its incorporated (active) NTZ.

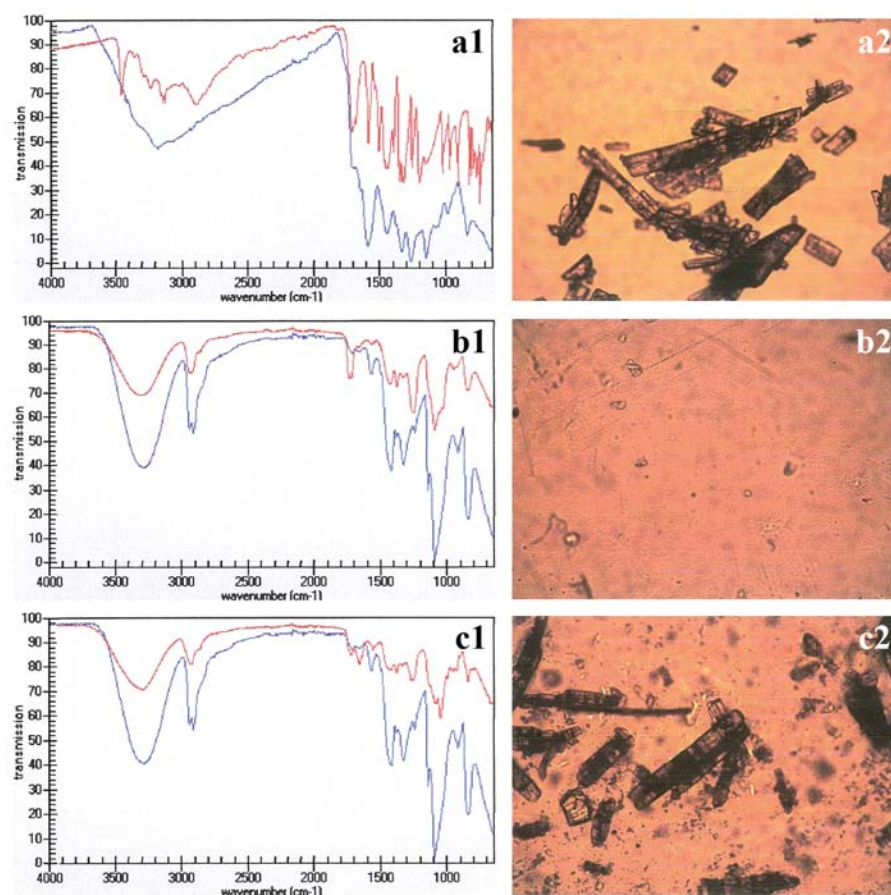


**Fig. (2).** Scanning electron microphotograph of the surface of the mucoadhesive polymeric film incorporating NTZ (magnification: x100).

The infrared spectra produced for plain nitrofurazone and for both plain hydrogels and hydrogels with NTZ incorporated are displayed in (Figs. **3a1**, **3b1** and **3c1**), respectively, and their magnifications (x36) displayed in (Figs. **3a2**, **3b2** and **3c2**), respectively. Infrared spectra of nitrofurazone was compared with the one for (standard) orcein dye, and the infrared spectra of hydrogels without (clPVA/Glut) and with (clPVA/Glut-NTZ) nitrofurazone were compared with that for hydrolyzed PVA. A careful inspection of (Fig. 3) may indicate that interaction between PVA and glutaraldehyde did occur. From comparison between the bands within 3550-3200  $\text{cm}^{-1}$  of the grouping (OH)OH...OH of intermolecular and intramolecular hydrogen bonds, one can notice a decrease of intensity which indicates a possible formation of acetal bridges. PVA presents another characteristic band at 2800-2695  $\text{cm}^{-1}$  from CH of aldehyde, which suffers interference after crosslinking with glutaraldehyde [22]. The other bands in 1461-1417  $\text{cm}^{-1}$  of the grouping (CH)-CH<sub>2</sub> and in 1366  $\text{cm}^{-1}$  of the grouping CH-R-CH<sub>2</sub> also displayed a decrease of intensity and may indicate bonding with glutaraldehyde. Comparison between (Figs. **3b1** and **3c1**) (infrared spectra of hydrogel without and with NTZ, respectively) shows no peak displacement at all, being comparable with the one from NTZ alone (see Fig. **3a1**). The spectrum profile

was fully conserved, which can most likely mean that NTZ virtually does not undergo interaction with the polymer upon its incorporation in the hydrogel, just being carried by the hydrogel system, which is in close agreement with the linear release profile observed for NTZ. For an inert hydrogel system, diffusion is the major force for drug uptake [32]. The diffusion of the drug through the gel matrix into the surrounding medium is controlled by the relaxation time of the polymeric chain, i.e. the time that the polymer takes to expand allowing the rapid diffusion of solute into the medium. The profile of the infrared spectra of hydrogels with and without nitrofurazone was maintained (see Fig. 3), which can mean that, probably, the interaction between NTZ and the polymer is not significative upon its incorporation into the hydrogel, and that it is just being carried by this hydrogel system. Thus, the release of NTZ into the medium was mainly influenced by the three-dimensional structure of the hydrogel and not by the crystal structure of nitrofurazone itself.

In studies involving thermogravimetric analyses (see Fig. 4), it has been observed that PVA exhibits two main regions of weight loss, 220-325°C and 400-425°C [33]. From inspection of the TGA analysis (see Fig. 4) plot of the hydrogel without



**Fig. (3).** Infrared spectra and images of (**a1** and **a2**, respectively) nitrofurazone (red line) compared with orcein dye (blue line, from data bank), infrared spectra and images of (**b1** and **b2**, respectively) hydrogel without nitrofurazone (clPVA/Glut, red line) and hydrolyzed PVA (blue line, from the data bank), and infrared spectra and images of (**c1** and **c2**, respectively) hydrogel with incorporated nitrofurazone (clPVA/Glut-NTZ, red line) and hydrolyzed PVA (blue line, from the data bank). [Processing parameters: Image (magnification, x36.0; width, 252.1  $\mu\text{m}$ ; height, 189.0  $\mu\text{m}$ ); experimental (analysis scans, 64; background scans, 64; resolution, a; spectral range, 4000.0-650.0; detector type, MCT; objective, 36X-ATR)].

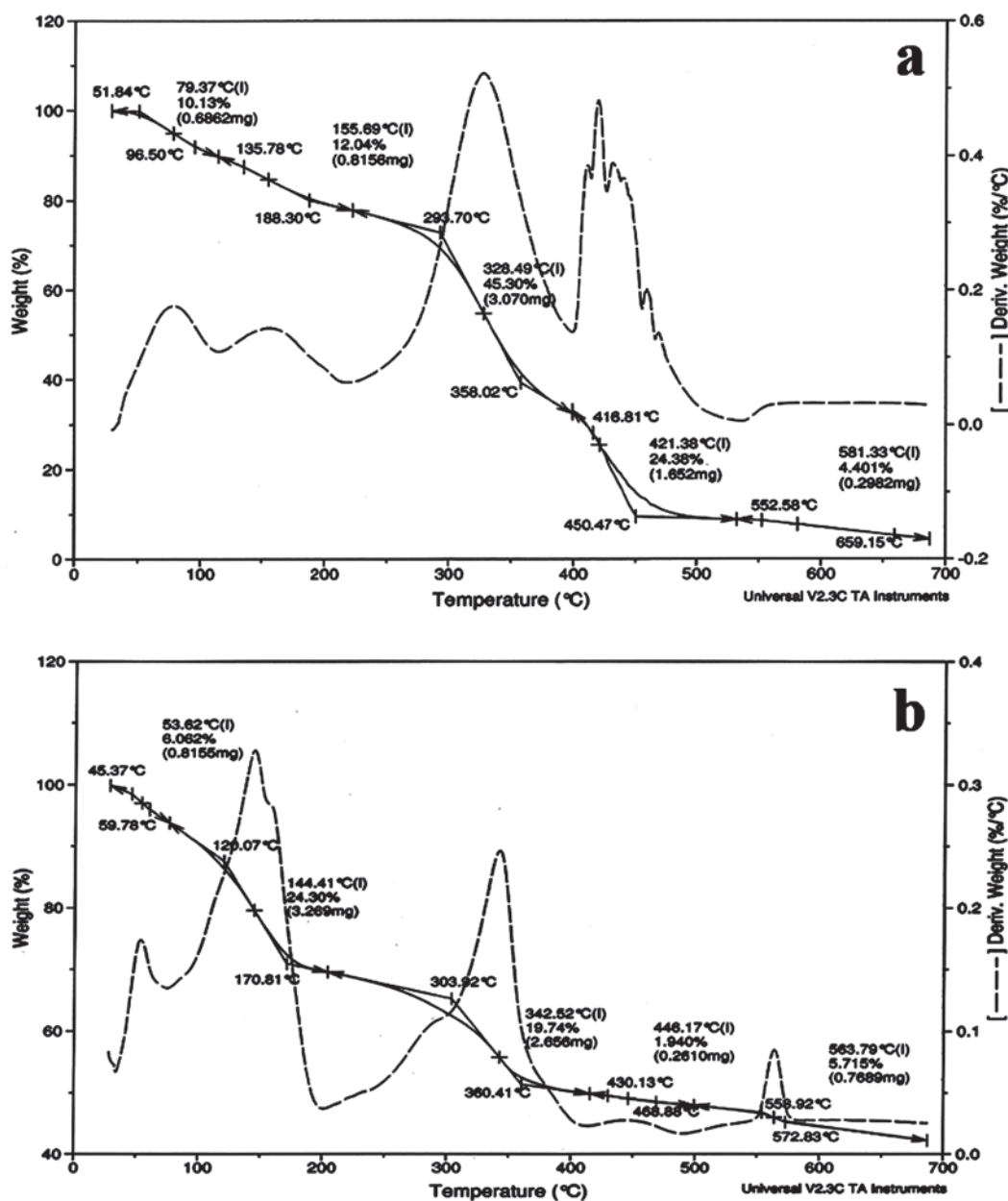
NTZ (cIPVA/Glut, see Fig. 4a), one observes a similar thermal behavior with 45.30% of weight loss at 328.5°C and with 24.38% of weight loss at 421.4°C. For the hydrogel with incorporated NTZ (cIPVA/Glut-NTZ, see Fig. 4b), significative weight losses were observed at 144.4°C (24.3%) and at 342.5°C (19.74%) (see Fig. 4b). These results may indicate that addition of NTZ suggest the miscibility and interphase interaction between the components of hydrogel. This miscibility can be attributed to hydrogen bonding between hydroxyl groups of PVA and the protonated nitro groups of NTZ.

These changes are an indication that the mechanisms of thermal degradation reactions of the PVA hydrogel crosslinked with glutaraldehyde (see Fig. 4a) are altered when NTZ is incorporated into the polymeric film (see Fig.

ure 4b). Meanwhile, the loss of thermal stability observed (see Figs. 4a,b) does not imply instability of the product for the intended use.

#### 4. CONCLUSION

The optimized hydrogel developed in this research effort presented an adequate appearance with respect to color, transparency and flexibility. These characteristics promote the acceptance of the product by the end users (and/or patients) and, most likely, the ease of application in different regions of the human body. The hydrogel exhibited good characteristics with respect to release of the active principle (NTZ), which were duly verified through swelling assays, *in vitro* NTZ release rates and via performance of (pathogenic)



**Fig. (4).** Thermogravimetric curves (TGA) of (a) plain hydrogel without NTZ (cIPVA/Glut) and (b) of the hydrogel with incorporated NTZ (cIPVA/Glut-NTZ).



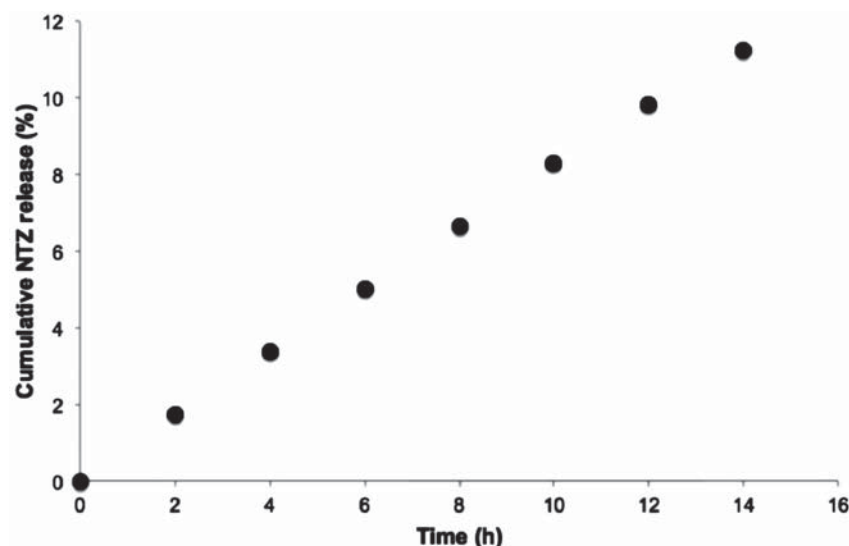


Fig. (5). Cumulative (*in vitro*) release profile of NTZ from the poly-(vinyl alcohol) hydrogel cross-linked with glutaraldehyde.

bacterial susceptibility tests. The swelling tests indicated an increase in weight of  $100 \pm 5\%$  up to  $350 \pm 11\%$  without loss of the original form, during a timeframe of assay of 14 h. The incorporated NTZ presented (as expected, relative to its susceptibility profile) antimicrobial activity. Regarding the *in vitro* NTZ release rate, the results obtained were highly satisfactory and pointed to a prolonged release of the drug. These characteristics may allow the use of the hydrogel in occlusive dressings for prolonged periods of time. The physico-chemical characterization of the hydrogels indicated obtention of a product with adequate structural characteristics. Scanning electron microscopy analyses allowed observation of a product with homogeneous morphology, and the infrared spectrophotometric analyses indicated that NTZ did not bind to the polymer in a way that would change its structure (which could reduce its antimicrobial activity). The thermal analyses have indicated that the hydrogel with incorporated NTZ may present extended thermal stability at the intended temperature of use ( $37^\circ\text{C}$  / body temperature). The ability of hydrogels to swell, under biological conditions, makes them an ideal material for use in drug delivery [34]. Due to their high water content, hydrogel creates a moist environment in the wound surface, which stimulates collagen synthesis and accelerates the growth and migration of epithelial cells. The hydrogel does not adhere to the wound surface and thus avoids the trauma during withdrawal [35]. Due to these reasons, the major goal of the research effort undertaken involved the development of a hydrogel with nitrofurazone as a thin film for wound treatment. From the results obtained in the research effort entertained herein, one may conclude that the hydrogel produced with NTZ presents promising characteristics that allows its use in the treatment of skin lesions, especially for patients with wounds difficult to treat as is the case of ulcerations of the lower limbs of patients with *Diabetes mellitus*.

#### CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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